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Discovery of Molecular Switches That Modulate Modes of Metabotropic Glutamate Receptor Subtype 5 ($mGlu_5$) Pharmacology in Vitro and in Vivo within a Series of Functionalized, Regioisomeric 2- and 5-(Phenylethynyl) pyrimidines

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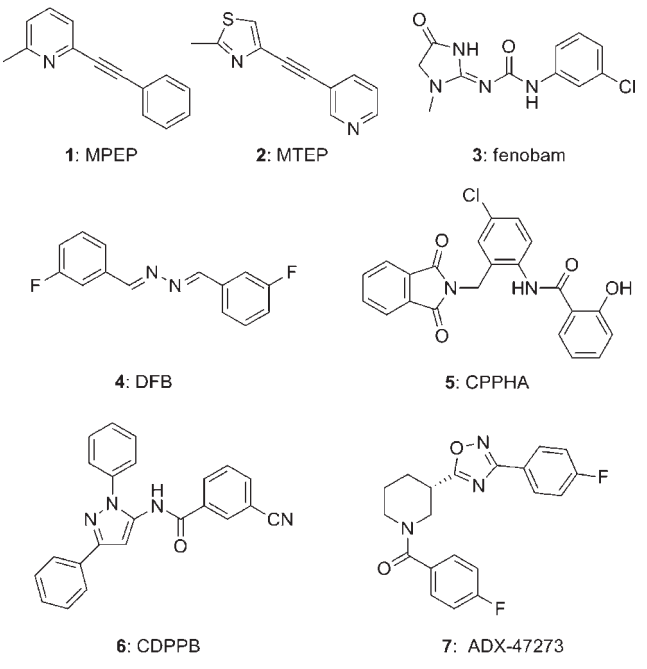
Abstract: We describe the synthesis and SAR of a series of analogues of the $mGlu_5$ partial antagonist 5-(phenylethynyl)pyrimidine. New molecular switches are identified that modulate the pharmacological activity of the lead compound. Slight structural modifications around the proximal pyrimidine ring change activity of the partial antagonist lead to that of potent and selective full negative allosteric modulators and positive allosteric modulators, which demonstrate in vivo efficacy in rodent models for anxiolytic and antipsychotic activity, respectively.

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system and exerts its effects through both ionotropic and metabotropic glutamate receptors. The metabotropic glutamate receptors (mGluRs[a]) are members of the G-protein-coupled recpetor (GPCR) family C, which are characterized by a large extracellular amino-terminal agonist-binding domain. To date, eight mGluRs have been cloned, sequenced, and assigned to three groups (group I, $mGlu_1$ and $mGlu_5$; group II, $mGlu_2$ and $mGlu_3$; group III, $mGlu_{4,6,7,8}$) based on their sequence homology, pharmacology, and coupling to effector mechanisms.[1,2] In preclinical models, studies with the negative allosteric modulators (NAMs) **1** (MPEP) and **2** (MTEP) (Chart 1) have demonstrated that selective antagonism of $mGlu_5$ has therapeutic potential for chronic disorders such as pain, anxiety, depression, addiction, and fragile X syndrome.[3−7] Furthermore, there is direct clinical validation of anxiolytic activity by allosteric antagonism of $mGlu_5$ in patients with fenobam **3**.[8] Alternatively receptor activity can be enhanced through positive allosteric modulators (PAMs) such as **4** (DFB), **5** (CPPHA), **6** (CDPPB), and **7** (ADX-47273), which with the exception of **5** share the same allosteric binding site as **1**.[9−13] PAMs **6** and **7**, both ago-potentiators, have demonstrated in vivo proof of concept in preclinical schizophrenia models in which other known antipsychotics show similar positive effects.[10−13] Recently, pure $mGlu_5$ PAMs have been developed based on **7**, by the incorporation of a basic heterocycle in the 3-position of the oxadiazole.[14] On the basis of our experience in the development of allosteric modulators of mGluRs with a broad range of activities including negative allosteric modulators, positive allosteric modulators and neutral allosteric site ligands at the allosteric binding site occupied by **1**, together with theoretical models of allosteric function, we postulated that it might be possible to develop "partial antagonists". As envisioned, a "partial antagonist" would fully occupy the binding site of **1** on the $mGlu_5$ receptor but only partially block agonist response, resulting in partial $mGlu_5$ inhibition; moreover, Rodriguez et al. identified several $mGlu_5$ partial antagonists.[15] In 2008, Sharma et al. conducted a limited optimization effort focused on the $mGlu_5$ partial antagonist lead **8**. Within two 24-member libraries, SAR elucidated a "molecular switch" to modulate pharmacological activity (Figure 1).[16] Lead **8**, with an unsubstituted distal phenyl ring, fully occupied the allosteric binding site of **1**, possessed an IC_{50} of 486 nM, but only afforded partial response (29% response, 71% partial antagonism), that is, allosteric partial antagonism. Incorporation of small chemical moieties in the 3-position of the distal phenyl ring, such as a 3-methyl group, delivered **9**, a full noncompetitive $mGlu_5$ antagonist (IC_{50} = 7.5 nM). When the methyl group was moved from the 3-position to the 4-position as in **10**, an efficacious (99% of glutamate max) $mGlu_5$ PAM resulted (EC_{50} = 3.3 μM, 4.2-fold shift), which also represented a new $mGlu_5$ PAM chemotype.[16] The observation of a conserved molecular switch, accessed by toggling between 3- and 4-substitution on the distal phenyl ring, within this chemical series was unprecedented. These preliminary data encouraged us to further optimize **8** and survey the impact of incorporating incorporating substituents on the pyrimidine ring, as well as examining regioisomeric pyrimidines to develop potent and

Chart 1. $mGlu_5$ Allosteric Ligands



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[a] Abbreviations: mGluR, metabotropic glutamate receptor; NAM, negative allosteric modulator; PAM, positive allosteric modulator; GPCR, G-protein-coupled receptor.

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selective $mGlu_5$ NAMs and PAMs suitable for in vivo studies to confirm the observed in vitro pharmacology.

For the next round of chemical lead optimization, we relied on an iterative analogue library synthesis approach[17] to rapidly prepare a 24-member library[18] in which 2-substituted-5-bromopyrimidines **11** were treated with phenylacetylene **13**, 3-methylphenyl acetylene (the NAM "switch") **14**, or 4-methylphenyl acetylene (the PAM "switch") **15** under microwave-assisted Sonogashira conditions (Scheme 1) to provide analogues **16**. In parallel, we prepared a small three-member library employing the regiosiomeric 2-bromopyrimidine **12** and **13**−**15** to deliver analogues **17**.

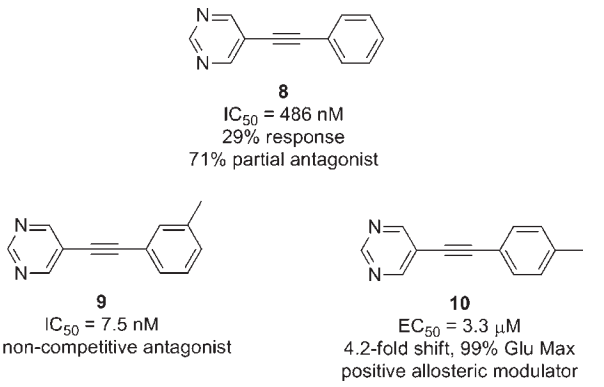
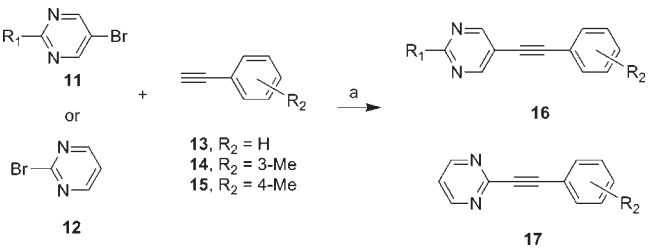


Figure 1. Identification of "molecular switches" that convert an $mGlu_5$ partial antagonist **8** to a full noncompetitive antagonist (NAM) **9** or a weak but fully efficacious $mGlu_5$ positive allosteric modulator (PAM) **10**.

Scheme 1. Synthesis of Analogues of **16** and **17**[a]

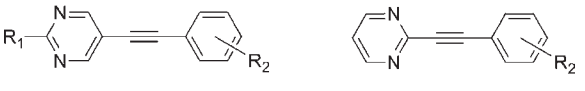


[a] Reagents and conditions: (a) 10 mol % $Pd(PPh_3)_4$, 20 mol % CuI, 20.0 equiv of diethylamine, DMF, microwave, 70 °C, 10 min, 16−95%; all compounds purified by mass-directed HPLC to >98% purity.[19]

SAR from this library was "flat", with few actives (Table 1); however, unexpected modulation of the mode of $mGlu_5$ pharmacology was observed. All new analogues **16** containing the 4-methylphenyl moiety were uniformly inactive, save for **16f**, a weak $mGlu_5$ PAM. When R_1 was an ethoxy group in combination with the NAM "switch", 3-methylphenyl, **16a** resulted, a potent $mGlu_5$ NAM (IC_{50} = 21 nM). The remaining analogues **16** were inactive or, more surprisingly, potent $mGlu_5$ PAMs. When an aminomethyl group was incorporated at the 2-position of the pyrimidine, in conjunction with an unsubstituted phenyl ring, **16b** resulted, which represents the most potent (EC_{50} = 14.3 nM, 15-fold shift) rat $mGlu_5$ PAM reported to date (10- to 15-fold more potent than **6** and **7**). Addition of the NAM "switch" 3-methylphenyl moiety with the 2-aminomethyl group **16c** unexpectedly afforded a similarly potent $mGlu_5$ PAM (EC_{50} = 21.1 nM, 5.9-fold shift), suggesting the 3-methylphenyl moiety is not a conserved molecular switch for engendering NAM activity. Interestingly, the NAM **16a** differs from the PAM **16c** by substitution at the 2-position of the pyrimidine, OEt versus NHMe, respectively, with equal potency (IC_{50} = 21 nM and EC_{50} = 21 nM, respectively) but opposite mode of pharmacology. Other groups were tolerated in the 2-position of the pyrimidine such as SMe **16d** and *t*-Bu **16e** and found to engender $mGlu_5$ PAM activity (EC_{50} = 120 nM, 11-fold shift and EC_{50} = 247 nM, 6-fold shift, respectively) but were inactive in the presence of the 3- or 4-Me-phenyl moieties. Overall, **16b** represents a 235-fold improvement in potency over $mGlu_5$ PAM **10**, was selective for mGlu5 (>10 μM vs $mGlu_{1-4,7,8}$), and warranted further evaluation.

The PAMs reported here demonstrated no activity in the absence of glutamate, but in the presence of a subthreshold concentration of glutamate (EC_{20}), a concentration dependent potentiation of $mGlu_5$ response was observed (Figure 2). Importantly, **16b** is a pure $mGlu_5$ PAM, not an ago-potentiator like **6** and **7**. In addition, **16b** demonstrated a robust 15-fold leftward shift of the glutamate concentration response

Table 1. Structures, Activity, and Mode of Pharmacology of Analogues **16** and **17**



	R_1	R_2	allosteric activity[a]	IC_{50}, EC_{50} (nM)[a]	antagonism (%)[a]	fold shift[a]
8	H	H	PA	486 ± 28	71	N/A
9	H	3-Me	NAM	7.5 ± 1.2	100	N/A
10	H	4-Me	PAM	3,300 ± 290	N/A	3.3
16a	OEt	3-Me	NAM	21.1 ± 2.8	100	N/A
16b	NHMe	H	PAM	14.3 ± 2.3	N/A	15
16c	NHMe	3-Me	PAM	21.1 ± 1.8	N/A	5.9
16d	SMe	H	PAM	120 ± 25	N/A	11
16e	*t*-Bu	H	PAM	247 ± 24	N/A	6.0
16f	NHMe	4-Me	PAM	704 ± 86	N/A	5.7
17a	N/A	H	NAM	195 ± 65	100	N/A
17b	N/A	3-Me	NAM	10.8 ± 2.1	100	N/A
17c	N/A	4-Me	N/A	>10000	N/A	N/A

[a] IC_{50}, EC_{50}, antagonism, and fold shift are the average of at least three independent determinations. N/A = not applicable. PA = partial antagonist. NAM = negative allosteric modualtor. PAM = positive allosteric modulator. Fold shift at 10 μM fixed concentration of compound.

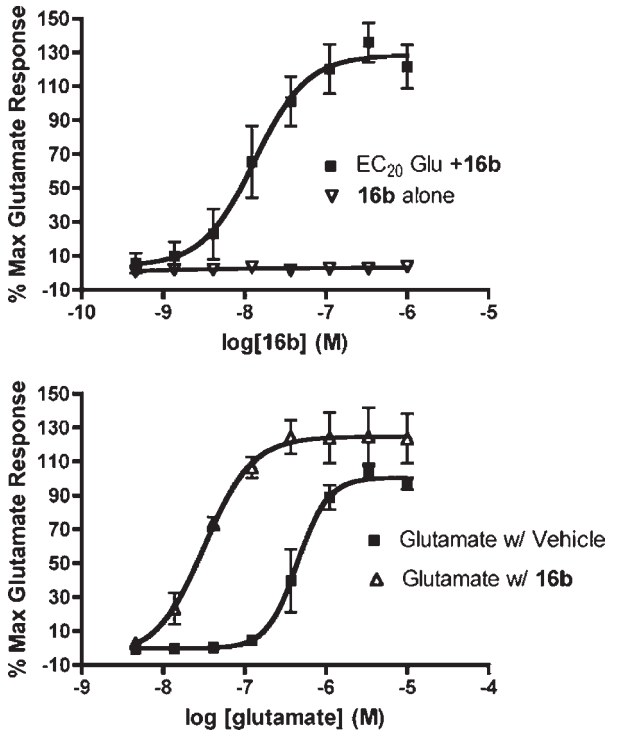


Figure 2. Compound **16b** potentiates $mGlu_5$ activation by glutamate. In the absence of glutamate, **16b** does not activate $mGlu_5$. In the presence of a subthreshold quantity of glutamate, **16b** potentiates $mGlu_5$ in a concentration-dependent manner. Compound **16b**'s potentiation of response to glutamate is manifested as increased $mGlu_5$ agonist sensitivity. The glutamate EC_{50} is shifted from 493 to 32 nM, or a 15-fold shift with 10 μM **16b**.

curve (EC_{50} shifts from 493 to 32 nM) with an increase in glutamate max (Figure 2).

In the regiosiomeric pyrimidine series **17**, the 4-Me congener **17c** was inactive. The unsubstitiuted phenyl analogue **17a** was a moderately potent $mGlu_5$ NAM ($IC_{50} = 195 \pm 65$ nM). Unlike series **16**, the 3-Me NAM "switch" performed as expected in series **17**, significantly increasing $mGlu_5$ NAM activity ($IC_{50} = 10.8 \pm 2.7$ nM) for **17b**. Moreover, **17b** was selective for $mGlu_5$ (> 10 μM vs $mGlu_{1-4,7,8}$).

With a potent $mGlu_5$ PAM **16b** and a potent $mGlu_5$ NAM **17b**, we were poised to determine if the modes of $mGlu_5$ modulation observed in our in vitro cellular assays would be mirrored in standard in vivo behavioral paradigms. To evaluate the PAM **16b**, we chose to study the ability of **16b** to reverse amphetamine-induced hyperlocomotion in rats, as **6** and **7** displayed robust efficacy in this preclinical model where other known antipsychotic agents show similar positive results.[10−13] In the event, **16b** was dosed ip at 3, 10, or 30 mg/kg 30 min prior to sc administration of 1 mg/kg amphetamine. As shown in Figure 3, a modest dose response is observed with **16b**, with significant reversal noted at the 30 mg/kg dose, and no effect (i.e, sedation) of **16b**/vehicle alone. Thus, the $mGlu_5$ PAM activity observed in cell-based in vitro assays is mirrored in vivo with **16b** and comparable to the effects seen with **6** and **7**.[10−13] Moreover, the reversal of amphetamine-induced hyperlocomotion with **16b** is important, as **16b** lacks the intrinsic agonism of the ago-potentiators **6** and **7**, suggesting for the first time that positive allosteric modulation alone is sufficient for an antipsychotic profile in this preclinical model.

Previously, $mGlu_5$ NAMs such as **1** and **2** have demonstrated anxiolytic activity in numerous preclinical models.

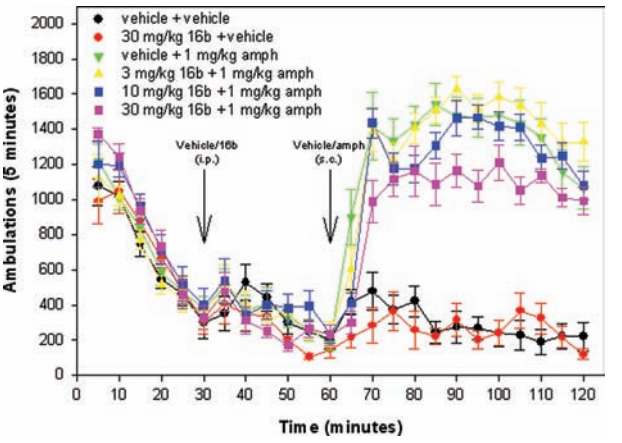


Figure 3. Reversal of amphetamine-induced hyperlocomotion with $mGlu_5$ PAM **16b** in dose-dependent manner with the nontoxic vehicle, 10% Tween-80.

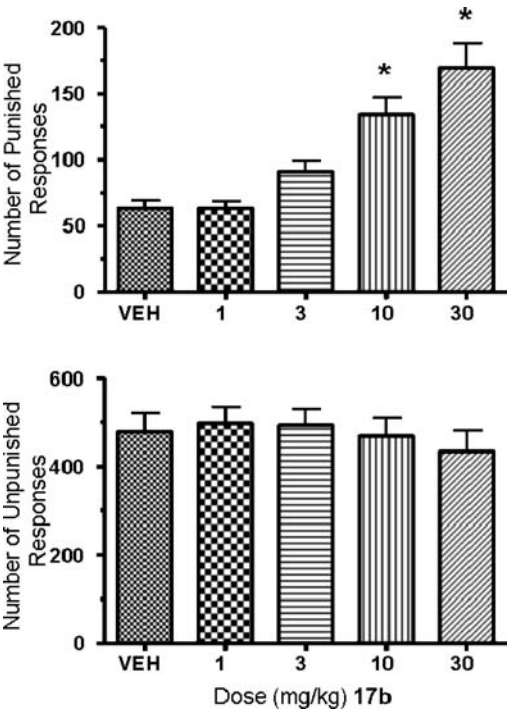


Figure 4. Dose−response curves for the effects of **17b** on punished (upper panel) and unpunished (lower panel) responding. The data are the mean number of punished and unpunished responses that animals made when tested on 1, 3, 10, 30 mg/kg **17b** and vehicle. Each value represents the mean ± SEM for 18 animals. For punished responding animals tested on 10 and 30 mg/kg **17b** made significantly greater number of responses than animals tested on vehicle ($p < 0.05$). Unpunished responding did not change significantly at any of the doses tested.

Therefore, **17b** was tested in a modified Geller−Seifter conflict model wherein an increase in punished responding is consistent with an anxiolytic-like profile.[20] As seen in Figure 4, **17b** produced a significant dose-dependent increase in punished responding with the 30 mg/kg dose approaching a 300% increase in response rate [$F(4,17) = 22.69$, $p < 0.0001$] (upper panel) with no significant effect on unpunished responding (lower panel). Post hoc analysis indicated that the 10 and 30 mg/kg doses in the punished component of the schedule differed significantly from vehicle ($p < 0.05$, Newman−Keul).

Therefore, the NAM activity observed in cell-based in vitro assays was again paralleled in a standard anxiolytic in vivo assay where classical $mGlu_5$ NAMs display similar positive results.[3−6,20]

In summary, slight structural changes to an $mGlu_5$ allosteric partial antagonist lead resulted in a shift in activity from partial antagonist to potent full antagonist to potent positive allosteric modulator. Two new molecular switches were elucidated through these changes. A regiosiomeric pyrimidine congener **17b** resulted in full NAM activity in vitro and in vivo. The incorporation of an amino methyl group into the 2-position of the pyrimidine core resulted in PAM activity, and this new molecular "switch" was able to override previously identified NAM molecular "switches". In this series, **16b** represents the most potent $mGlu_5$ PAM reported to date and the first example of in vivo efficacy of a pure $mGlu_5$ PAM in reversing amphetamine-induced hyperlocomotion. The resulting $mGlu_5$ NAM **17b** and PAM **16b** showed in vivo efficacy in rodent models of anxiety and schizophrenia, respectively, which mirrored the observed in vitro mode of pharmacology. With such subtle structural modifications capable of fully reversing modes of pharmacology, lead optimization campaigns focused on ligands that bind to the allosteric site occupied by **1** are especially challenging. Further work in this area is in progress and will be reported in due course.

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Supporting Information Available: Experimental procedure and analytical data for **16a**−**f** and **17a**−**c**; details of the in vitro and in vivo assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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